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ISSN: 2249-3875

International Journal of Pharmaceutical Erudition

Research for Present and Next Generation

ISSN 2249-3875

International Journal of Pharmaceutical Erudition

Research paper

ANTI FERTILITY ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF *TRILLIUM GOVANIANUM* IN ETHINYL ESTRADIOL INDUCED ANTI FERTILITY MODEL IN RATS

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Lack of fertility is in fertility were as anti-fertility is to reduce fertility. Fertility is burning issue of global as well as national public issue which are concerning the fast growth of the country. Increase rate of population has got and adverse effect on the economy of the countries. So there is an urgent and important need for the anti fertility services in the country. In present study anti implantation and anti estrogenic activity of hydro alcoholic extract of *Trillium govanianum* was evaluated in Ethinyl estradiol induced anti fertility model. Obtained result suggests that *Trillium govanianum* at the doses of 250 and 500 mg/kg body weight produced a dose dependent adverse effect on fertility index and number of implantation in the uterine horns of the female rats by virtue of an increase in the percentage of the post implantation embryonic loss. All the experimental extract when evaluated for their abortifacient activity, were found to exhibit pregnancy interceptive activity. Administration of 100 mg/kg body weight of the alcoholic extract resulted in 100% abortion, while doses of 250 and 500 mg/ kg body weight of the alcoholic extract resulted in 28.50% and 44.45% abortion. This was evident from decrease in the percentage of live fetuses. The percent resorption index increased from zero in the control animals to 100 % in 100 mg/kg body weight alcoholic extract treated animals.

KEYWORDS- anti-fertility *Trillium govanianum*, Ethinyl estradiol , anti fertility model.

INTRODUCTION

To become parent is one of the most universally desire goal in adulthood, and every one dream to have life that have children, but not all couple will achieve this goal due to Various reasons. As we know that fertility is a nature phenomenon and is define as the capability to produce offspring. Lack of fertility is in fertility were as anti-fertility is to reduce fertility. Fertility is burning issue of global as well as national public issue which are concerning the fast growth of the country. Increase rate of population has got and adverse effect on the economy of the countries. So there is an urgent and important need for the anti fertility services in the country. Anti-fertility is the preventive method to help women to avoid unwanted pregnancies which include all the temporary as well as permanent measure to prevent pregnancy resulting from intercourse. Anti-fertility methods are agents are safe, effective, reversible and last lasting. Now it has become important to use anti-fertility agents are methods which can interfere with the natural procedure of reproduction in women. Over population throughout the world has

adverse effect on life supporting system. So human fertility must be controlled to control the population and anti-fertility play a significant role in doing so on.

Mechanism

Anti-fertility agents act by disrupting pre-ovulatory and pre-implantation phases. They show their effect in number of areas in female body, Hypothalamus, anterior pituitary, ovary-oviduct, uterus, cervix and vagina. Anti-fertility agents that prevent ovulation and fertilization are commonly called as contraceptives and those use after implantation are called abortifacients. Anti-implantation activity is due to estrogen activity by expulsion of ova from the tube, Disrupting the leteotropic activity of the blastocyst by disturbing the estrogen and progesterone equilibrium or by creating non-receptive condition in the uterus hence prevent ovulation result in the prevention of fertilization.

Plants used for anti-fertility activity

World health organisation estimates that 80% of the world population relies on herbal medicines. Synthetic drugs may cause and undesirable effects· Were as natural products are considered safe and effective, Herbal medicines are popular for improving life with no side effects. As we know cancer is second leading cause of death worldwide. Natural therapies such as use of plant derived products in cancer treatment may act as boon. Currently few plants and their products are used to treat cancer.

MATERIAL AND METHODS:

Abortifacient activity

The female rats in proestrous phase were caged with males of proven fertility in the ratio of 2:1, in the evening and examined the following day for the evidence of copulation.

Rats exhibiting thick clump of spermatozoa in their vaginal smear were separated and that day was designated as day 1 of pregnancy. Selected animals were divided into three groups, consisting of six rats in each group.

Group I served as control and received vehicle only (Tween-80)

Group II received *Trillium govanianum* 250 mg/kg from day 8th to 14th.

Group III received *Trillium govanianum* 500 mg/kg from day 8th to 14th

On the day 10 of pregnancy animals were laparotomies under light ether anesthesia using sterile conditions. The two horns of uteri were examined to determine the implantation sites. Thereafter the abdominal wound was sutured in layers.

During the experiment animals were observed for vaginal bleeding. The animals were allowed to go full term. After delivery the pups were counted and the abortifacient activity of extract was evaluated by computing parameters like litter size and resorption index.

Estrous cycle study

Female rats (200–250 g) showing normal estrous cycle were selected and divided into three groups of six animals each.

Group I served as control and received vehicle orally for thirty days.

Group II received 250 doses of *Trillium govanianum*

respectively by oral route daily for same period.

Group III received 500 mg/kg doses of *Trillium govanianum* respectively by oral route daily for same period.

The vaginal smears were observed every morning in all the three groups of animals to check any variation in proestrous, estrous, metaestrous and diestrous phase.

Estrogenic/anti-estrogenic activity

Female Wistar rats (200–250 g) were bilaterally ovariectomised under light ether anesthesia and semi-sterile conditions. After one week, they were divided into six groups consisting of 6 animals each.

Group I (control) was administered with vehicle (Tween-80, 5% v/v).

Group II (standard) received standard drug EE (1 µ/rat/day, s.c) suspended in olive oil.

Groups III received only hydro alcoholic extract of *Trillium govanianum* at doses of 250 mg/kg

Groups -IV received only hydro alcoholic extract *Trillium govanianum* at doses of 500 mg/kg.

Groups V received *Trillium govanianum* at doses of 250mg/kg along with EE (1 µ/rat/day, s.c) for 7 consecutive days.

Groups VI received *Trillium govanianum* at doses of 500mg/kg along with EE (1 µ/rat/day, s.c) for 7 consecutive days.

On the 8th day final body weight of all animals was measured. Then all animals were sacrificed under light anesthesia. Uterine weight, vaginal opening and cornification of all the animals were observed and blood serum was further processed for the estimation of biochemical parameters

Histology of uterus

On the 15th day after treatment with hydro alcoholic extract of *Trillium govanianum* at doses of 250mg/kg and 500 mg/kg the female rat uterus were extract out and examined for histological parameters.

RESULT AND DISCUSSION:

The female rats in proestrous phase were caged with males of proven fertility in the ratio of 2:1, in the evening and examined the following day for the evidence of copulation.

Rats exhibiting thick clump of spermatozoa in their vaginal smear were separated and that day was designated as day 1 of pregnancy. Selected animals were divided into three groups, consisting of six rats in each group.

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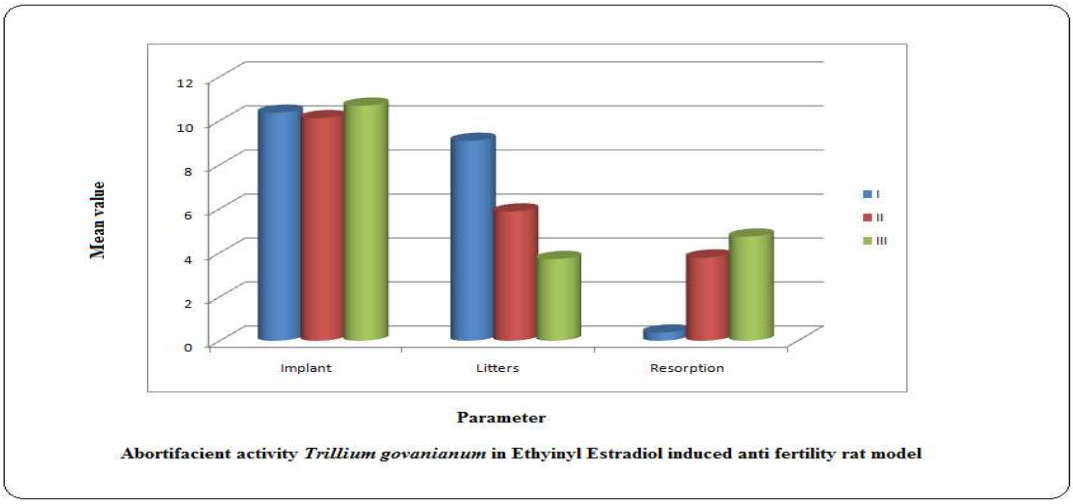
During the experiment animals were observed for vaginal bleeding. The animals were allowed to go full term. After delivery the pups were counted and the abortifacient

ISSN 2249-3875

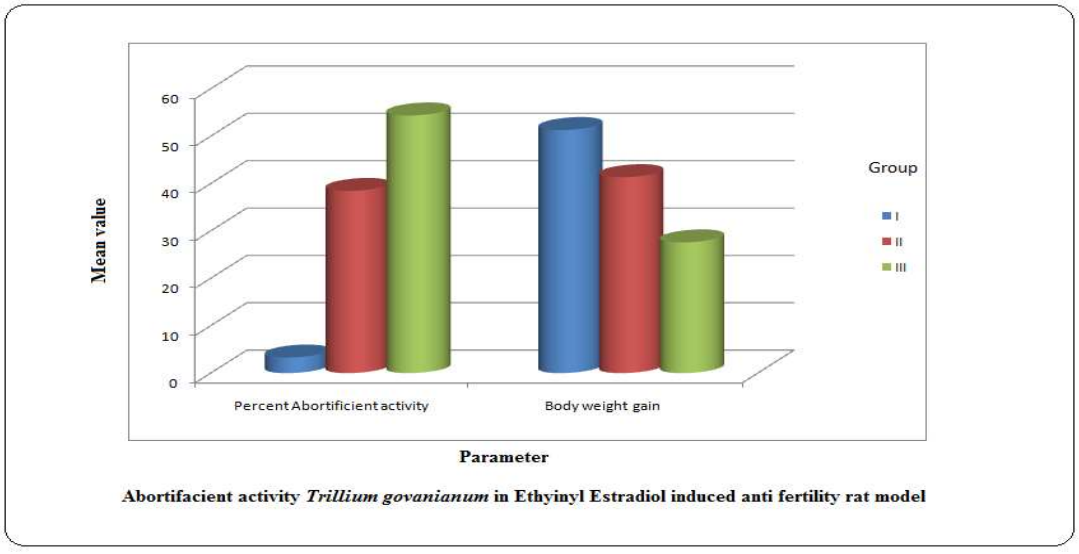
International Journal of Pharmaceutical Erudition

Table 1 : Abortifacient activity *Trillium govanianum* in Ethyinyl Estradiol induced anti fertility rat model

Group	No of Pregnant females (n)	Mean ± SEM			% Abortificient activity	Body weight gain
		Implant	Litters	Resorption		
I	6	10.36 ± 0.76	9.08 ± 0.27	0.37 ± 0.38	3.28 %	51.3 ± 0.98
II	6	10.11 ± 0.76	5.87 ± 0.24	3.78 ± 0.57	38.45 %	41.35 ± 0.15
III	6	10.68 ± 0.23	3.71 ±0.17	4.73 ± 0.41	54.38 %	27.59 ± 0.57



Histogram 1: Abortifacient activity *Trillium govanianum* in Ethyinyl Estradiol induced anti fertility rat model



Histogram 2: Abortifacient activity *Trillium govanianum* in Ethyinyl Estradiol induced anti fertility rat model

activity of extract was evaluated by computing parameters like litter size and resorption index. The results are summarized in **Table 1** and **Histogram 1** and **2**

Effect of of *Trillium govanianum* on Estrous cycle

Female rats (200–250 g) showing normal estrous cycle were selected and divided into three groups of six animals each.

Group I served as control and received vehicle orally for thirty days.

Group II received 250 doses of *Trillium govanianum* (TG)respectively by oral route daily for same period.

Group III received 500 mg/kg doses of *Trillium govanianum* (TG)respectively by oral route daily for same period.

The vaginal smears were observed every morning in all the three groups of animals to check any variation in proestrous, estrous, metaestrous and diestrous phase. The results are summarized in **Table 2** and **Histogram 3**

Estrogenic/anti-estrogenic activity of *Trillium govanianum*

Female Wistar rats (200–250 g) were bilaterally ovariectomised under light ether anesthesia and semi-sterile conditions. After one week, they were divided into six groups consisting of 6 animals each.

Group I (control) was administered with vehicle (Tween-80, 5% v/v).

Group II (standard) received standard drug EE (1 µ/rat/day, s.c) suspended in olive oil.

Groups III received only hydro alcoholic extract of *Trillium govanianum* at doses of 250 mg/kg

Groups -IV received only hydro alcoholic extract *Trillium govanianum* at doses of 500 mg/kg.

Groups V received *Trillium govanianum* at doses of 250mg/kg along with EE (1 µ/rat/day, s.c) for 7 consecutive days.

Groups VI received *Trillium govanianum* at doses of 500mg/kg along with EE (1 µ/rat/day, s.c) for 7 consecutive days.

The results are summarized in **Table 3** and **Histogram 4** and **5**

Table 2: Effect of of *Trillium govanianum* on Estrous cycle in anti fertility rat model

Estrous Cycle	Control	TG 250 mg/kg	TG 50 mg/kg	Vaginal Opening	Cell type
Proestrus	12.3 ± 0.02	5.8 ± 0.03	5.12 ± 0.02	Nil	Epithelial cell
Estrous	14.6 ± 0.05	7.3 ± 0.06	6.47 ± 0.04	Approximate 50%	Cornfied cell
Metaestrus	22.49 ± 0.04	12.48 ± 0.03	10.98 ± 0.02	More than 50%	Cornified cell + Leukocyte
Diestrus	57 ± 0.02	122 ± 0.07	129 ± 0.03	Approximate 60%	Leukocyte + epithelial cell

ISSN 2249-3875

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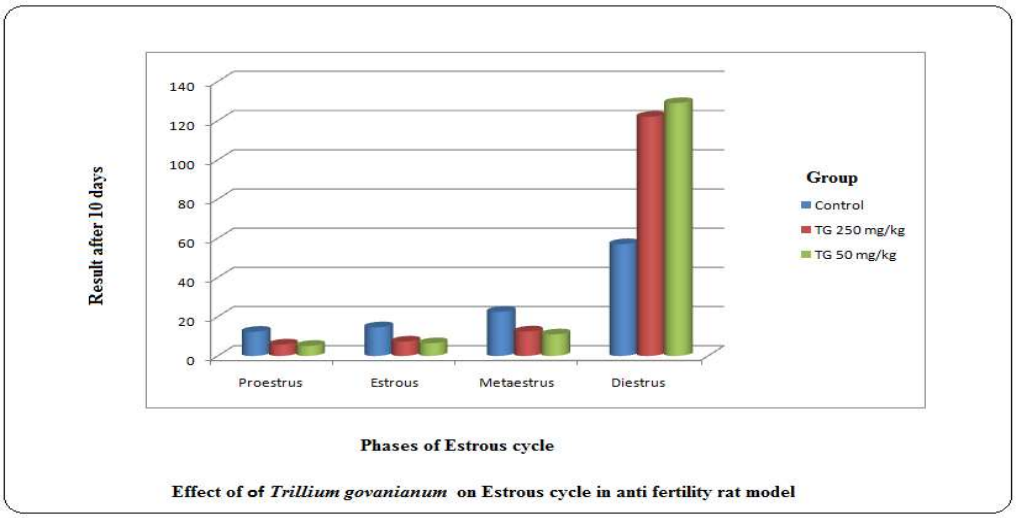
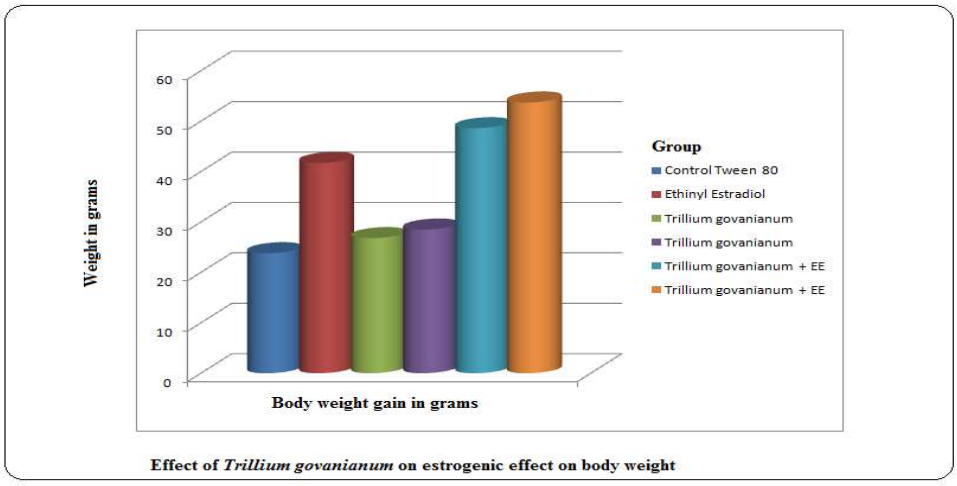


Histogram 3: Effect of *Trillium govanianum* on Estrous cycle in anti fertility rat model

Table 3: Effect of *Trillium govanianum* on estrogenic effect on body weight and uterine weight in female rats

Treatment (Route)	Dose (mg/kg)	Body weight gain (g)	Uterine weight (g)
Control Tween 80	2% v/v	23.8 ± 2.8	0.92 ± 0.002
Ethinyl Estradiol (s.c.)	1 µg/rat	41.7 ± 4.09	1.03 ± 0.005
Trillium govanianum	250 mg/kg	26.8 ± 3.7	1.04 ± 0.002
Trillium govanianum	500 mg/kg	28.5 ± 2.6	1.16 ± 0.008
Trillium govanianum + EE	250mg/kg	48.6 ± 1.32	1.39 ± 0.006
Trillium govanianum + EE	500 mg/kg	53.7 ± 2.16	1.57 ± 0.005



Histogram 4: Effect of *Trillium govanianum* on estrogenic effect on body weight

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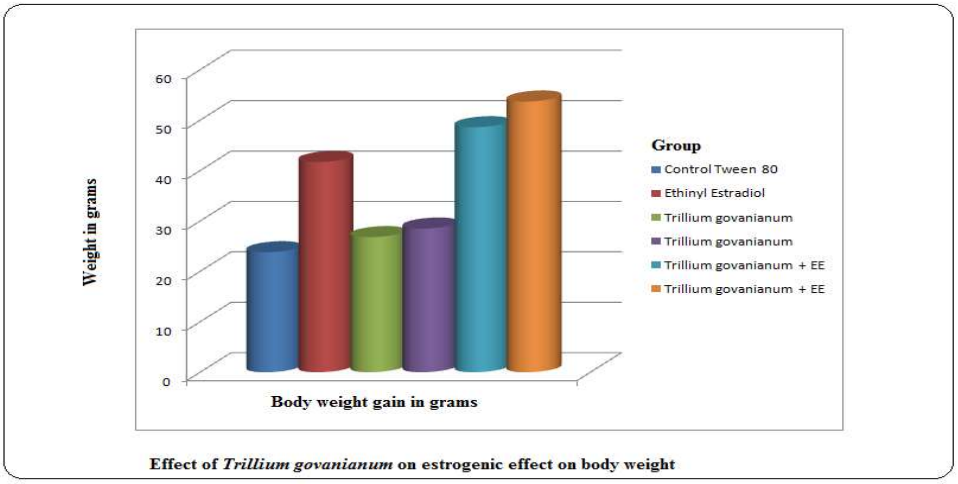
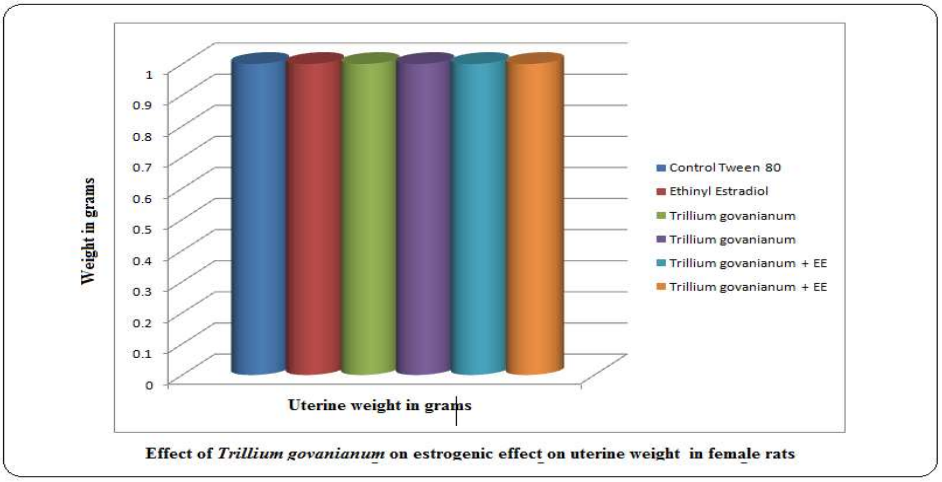


Histogram 4: Effect of *Trillium govanianum* on estrogenic effect on body weight



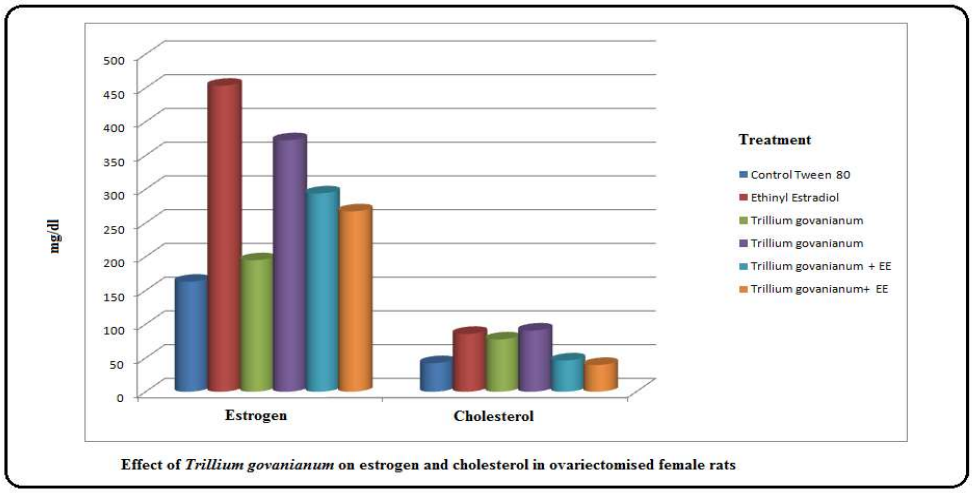
Histogram 5: Effect of *Trillium govanianum* on estrogenic effect on uterine weight

Table 4: Effect of *Trillium govanianum* on biochemical parameters in ovariectomised female rats

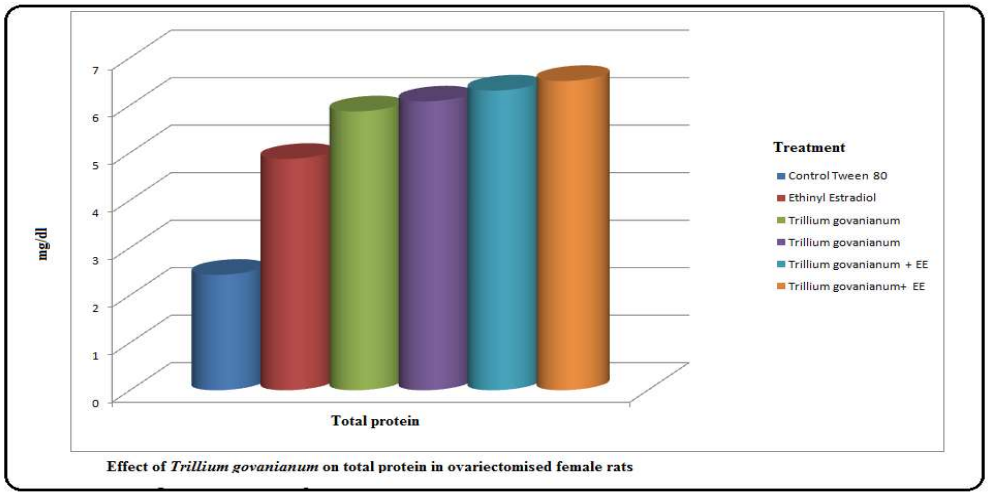
Treatment	Dose	Total Protein content	Estrogen (pg/ml)	Cholesterol (mg/dl)
Control Tween 80	2% v/v	2.43 ± 0.4	163 ± 3.01	42.38 ± 1.68
Ethinyl Estradiol (s.c.)	1 µg/rat	4.87 ± 0.49	453.29 ± 3.49	85.72 ± 1.86
Trillium govanianum	250 mg/kg	5.87 ± 0.73	194.84 ± 3.82	77.58 ± 1.46
Trillium govanianum	500 mg/kg	6.08 ± 0.94	372.63 ± 2.04	90.73 ± 1.83
Trillium govanianum + EE	250 mg/kg	6.31 ± 0.57	293.82 ± 2.62	46.38 ± 1.47
Trillium govanianum + EE	500 mg/kg	6.51 ± 0.81	267.30 ± 3.01	39.71 ± 1.93

ISSN 2249-3875

International Journal of Pharmaceutical Erudition



Histogram 6: Effect of *Trillium govanianum* on estrogen and cholesterol in ovariectomise



Histogram 7: Effect of *Trillium govanianum* on total protein in ovariectomised female rats

Table-5: Effect of *Trillium govanianum* on endometrium dimensions in ovariectomised female rats

Treatment	Dose	Diameter of uterus (µm)	Thickness of endometrium (µm)	Height of endometrium
Control Tween 80	2% v/v	341.08 ± 0.2	54.2 ± 0.22	12.29 ± 0.75
Ethinyl Estradiol (s.c.)	1 µg/rat	612.93 ± 0.91	241.06 ± 1.08	33.12 ± 0.88
Trillium govanianum	250 mg/kg	381.71 ± 0.59	71.92 ± 1.68	18.06 ± 0.31
Trillium govanianum	500 mg/kg	390.82 ± 1.62	69.50 ± 0.83	24.68 ± 1.04
Trillium govanianum + EE	250 mg/kg	512.03 ± 1.53	196.81 ± 1.45	18.13 ± 0.61
Trillium govanianum + EE	500 mg/kg	601.78 ± 1.04	204.13 ± 1.36	33.54 ± 0.47

ISSN 2249-3875

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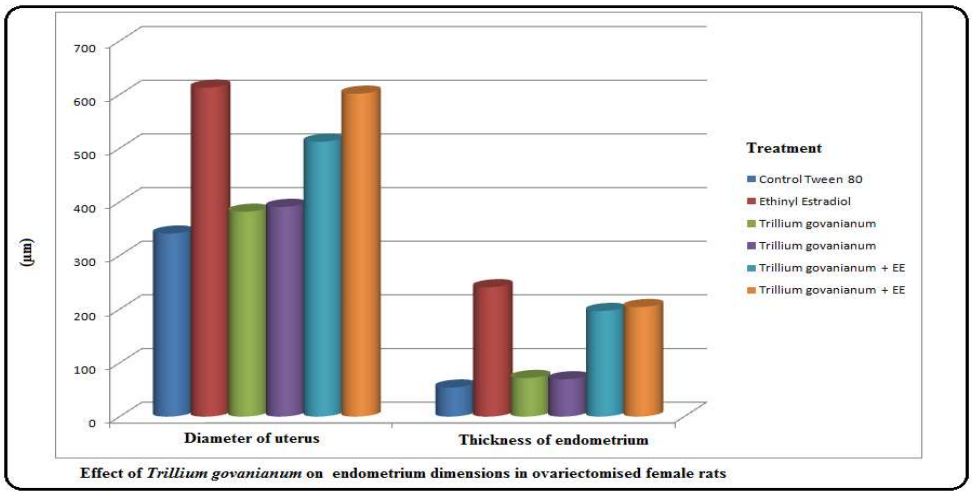
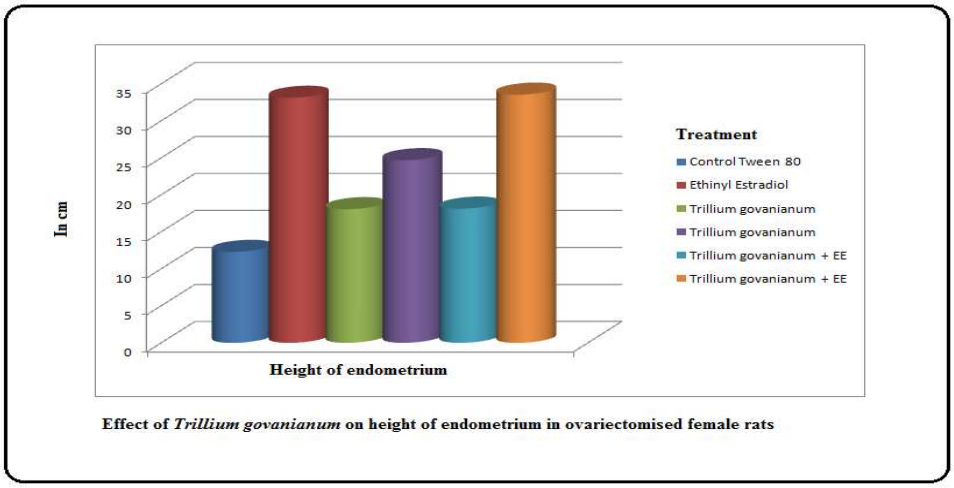


Histogram 8: Effect of *Trillium govanianum* on endometrium dimensions in ovariectomised female rats



Histogram 9: Effect of *Trillium govanianum* on height of endometrium in ovariectomised female rats

SUMMARY AND CONCLUSION:

The present work also corroborates 100% abortive effect of ethanol extract of stem bark of Trillium govanianum at a dose of 100mg/kg body weight. The antifertility activity of 50 % ethanolic extract of Trillium govanianum excluding root was demonstrated in hamstar. The antifertility activity of 50 % ethanolic root extract of Trillium govanianum was investigated and it was found that a dose of 200 mg/kg led to foetal resorption in 60 % female pregnant rats. All the treatment reduced significantly the number of litters born, confirming the abortifacient activity of the plant used. No vaginal bleeding was observed. The litter born to the experimental animal did not show any morphological defects hence, it can be stated that the treatment does

ISSN 2249-3875

International Journal of Pharmaceutical Erudition

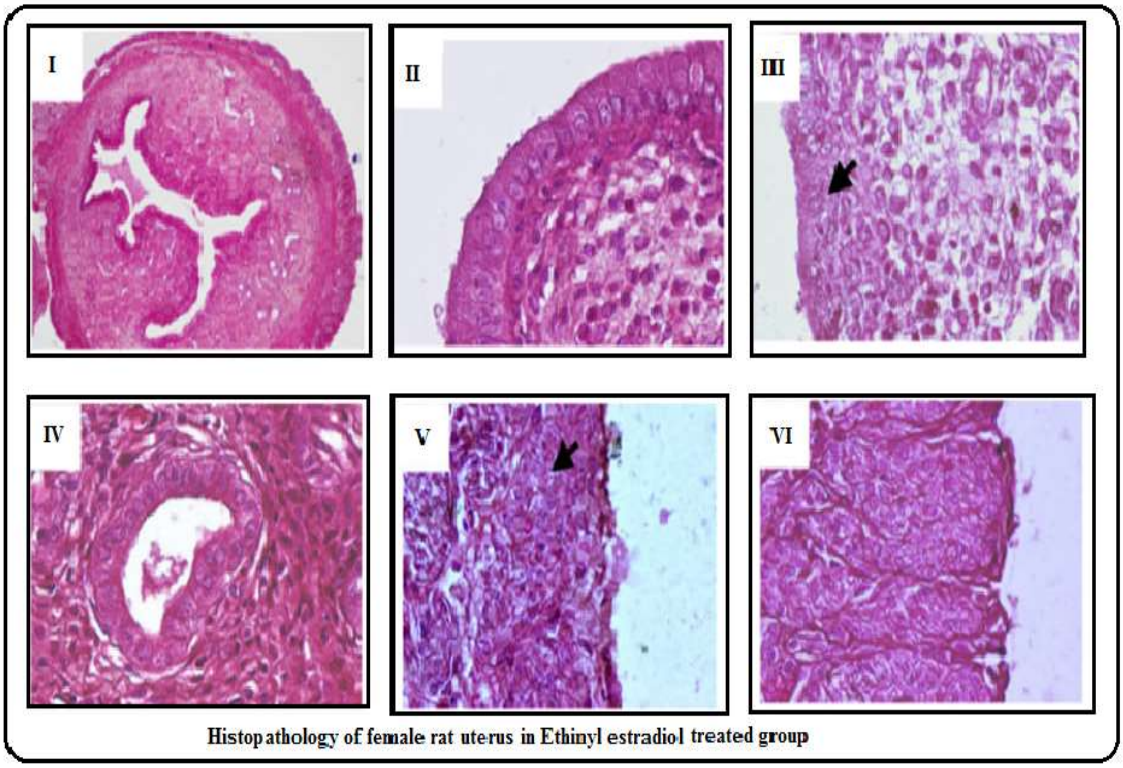


FIG. 1: HISTOPATHOLOGICAL EXAMINATION OF RAT UTERUS

not exhibit any teratogenic effect. The ethanolic extract ofTrillium govanianum stem bark at the dose of 100 mg/kg body weight exhibited significant abortifacient activity hence it was further selected for confirmation of the antifertility activity of the plant. In the estrogenic study, the effect of alcohol extract of Trillium govanianum stem bark revealed that none of the control group none of the rats exhibited vaginal opening during the period of study. The alcohol extract at the dose of 100 mg/kg when administered orally for 7 days, showed vaginal cornification in all the animals and also increased the uterine weight (P< 0.01) when compared with control, but the extent of the uterotropic response was less than that produced by ethinyl estradiol alone.

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